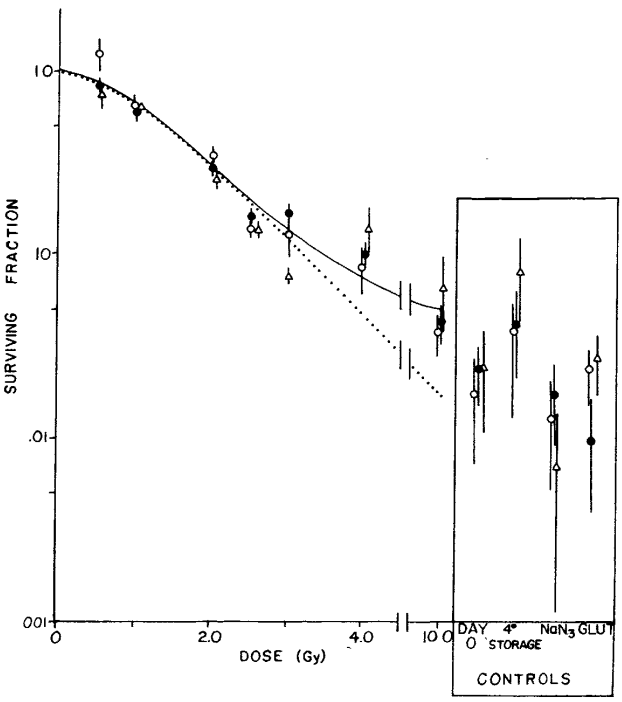
## RADIATION SENSITIVITY OF CLONOGENIC HUMAN MELANOMA CELLS

SIR,—A widely used bioassay of clonogenic cells from human tumours is the one developed by Hamburger and Salmon.[1] A major criticism of its use for assessing the drug sensitivity of a tumour has been the lack of evidence for survival curves after ionising radiation that would be consistent with earlier experience with mammalian cell lines.[2,3] My preliminary studies suggested that clonogenic human melanoma cells exhibited marked radioresistance.[4] These results on radiation dosage and survival of melanoma colony-forming cells may have been due to cellular aggregates present at the start of culture.

The general culture method has been described in detail elsewhere.[1,5] Melanoma tumour cells from biopsy material were diluted in plating medium and incubated at 37°C for 2·0 h. The cell suspension was put into plastic tubes and irradiated to varying doses (0·5, 1·0, 2·0, 2·5, 3·0, 4·0, 10·0 Gy) at a dose rate of 5·0 Gy/min. The cells were plated into the culture system within an hour of irradiation. Most investigators are aware of the difficulty in obtaining single-cell preparations, so, besides the experimental controls, "cellular aggregate" controls were used to assess the single-cell nature of the experimental plates. These controls included: (1) counting of plates 1 h after plating (day 0); (2) storage of plates at 4°C; (3) treatment with 1·0% sodium azide; and (4) fixation of plates in 3% glutaraldehyde. Plates treated with conditions 3 and 4 were incubated along with experimental plates. Under conditions 2, 3, and 4, aggregates should be preserved and cellular proliferation stopped. These controls were counted at the completion of the experiment.

The relation between survival of melanoma colony-forming cells and dose of radiation for a representative patient is shown in the figure. The uncorrected curve (solid line) for colony number showed a shoulder and a log-linear decrease in survival, but a plateau was evident. The different cellular aggregate controls produced colony counts similar to those for the 10·0 Gy dose, so the mean of the different cellular aggregate controls was subtracted from the experimental data to produce the curve shown by the dotted line. The $D_0$ was 2·38 Gy. Clonogenic melanoma cells from two other patients and from a murine melanoma (CCL 53·1) cell line were also studied. In case 2 a shoulder was evident, and the $D_0$ was 2·60 Gy. In the third case and in cells from the murine line, no shoulder was present, and $D_0$ values of 3·76 and 4·10 were calculated, respectively. No differential effect was noted on survival of colony-forming cells giving rise to colonies of different sizes in any of these experiments. The shapes of the survival curves and the $D_0$ values closely resemble those from other tumour cell types, as well as for both human and murine melanoma cells using other clonogenic assays.[6,7]

The demonstration that tumour specimens with apparent "plateaus" display a single negative exponential radiation survival curve when background clumping is subtracted raises serious questions about the validity of previous assessments of in vitro chemosensitivity analysis using this agar culture system. It is possible that the relatively positive correlations of in vitro and in vivo results that have been reported with this assay, despite the demonstration of artificially induced changes in survival curves, are related to the fact that the portion of the survival curve analysed for drug sensitivity is largely the first third of the survival curve, therefore minimising variation from cell clumping (ie, artificial plateaus) present at the end of the survival curve.




**Radiation survival curve for clonogenic human cells (81–61).**

Solid line = uncorrected; dotted line = mean of cellular aggregate controls (see text) subtracted from experimental plates. Six replicates for each experimental plate were used. Standard errors are included. Colonies with diameter (μm) 50–60 (●), 60–72(○), 72–86 (Δ).

Increasing evidence indicates that the assay does reflect growth of human tumour stems cells[8,9] and suggests that this approach is conceptually sound.[3,6] Therefore, as suggested by Hill,[10] we should be encouraged to improve the methodology in this important and exciting area of cancer research.

Supported in part by grants from the American Cancer Society (PDT 184) and the National Cancer Instutite (CA17094).

Department of Internal Medicine
and Cancer Center,
University of Arizona Health Sciences Center,
Tucson, Arizona 85724, USA

FRANK L. MEYSKENS, JR

1 Hamburger AW, Salmon SE. Primary bioassay of human tumour stem cells. *Science* 1977, **197:** 461–63
2 Editorial. Clonogenic assays for the chemotherapeutic sensitivity of human tumours. *Lancet* 1982, i 780–81.
3 Selby P, Buick RN, Tannock I. A critical appraisal of the "human tumor stem cell assay" *N Engl J Med* 1983; **308:** 129–34.
4 Meyskens FL Human melanoma colony formation in vitro. In: Salmon S, ed. Human tumor cloning in vitro. *Progr Clin Biol Res* 1980; **48:** 85–99.
5 Meyskens FL Jr, Soehnlen B, Saxe DF, Casey WJ, Salmon SE. In vitro clonal assay for human metastatic melanoma cells. *Stem Cells* 1981; **1:** 61–72.
6 Steel G Growth and survival of tumour stem cells. In: Steel G, ed. Growth kinetics of tumour cells Oxford. Clarendon Press, 1977. 254–257.
7 Selby PJ, Courtenay VD In vitro cellular radiosensitivity of human malignant melanoma *Int J Rad Oncol Biol Phys* 1982; **8:** 1235–1237.